Vol. XXIX *August, 1915.* *No. 2*

BIOLOGICAL BULLETIN

SEXUAL REACTIONS BETWEEN HERMAPHRODITIC AND DIŒCIOUS MUCORS.[1]

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INTRODUCTION.

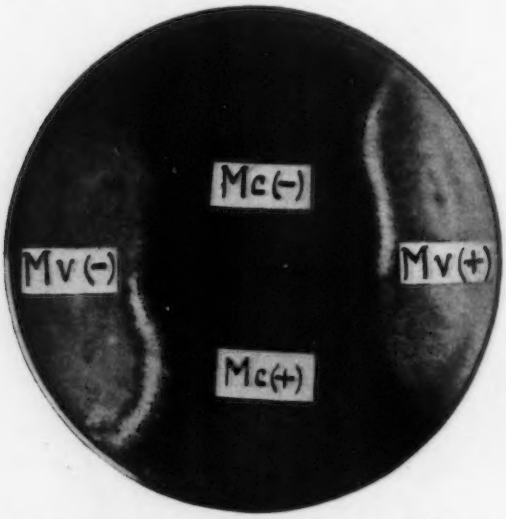
Conjugation among the Mucors has been assumed to represent the simplest type of reproduction acknowledgedly sexual in character. Some even have denied the term sexual to the union of morphologically similar gametes such as occur in this group. The present article will present further evidence in favor of the author's contention that although the process is morphologically a simple one, conjugation in the Mucors is as definitely a sexual process as the morphologically more complex type of reproduction in higher forms and that the sexes seem even more sharply distinct.

It has been shown by the writer ('04, '09) that the majority of the forms among the Mucors are diœcious, with the sexes separated in male and female races which are capable of being propagated apparently to an indefinite number of vegetative generations by means of nonsexual spores formed in sporangia. In all the diœcious species carefully investigated the opposite gametes, which are produced and unite to form zygospores when the two sexual races of a given form are grown together, do not appear to differ morphologically. Lacking a definite criterion which an inequality of the gametes would have afforded, the writer has provisionally designated the opposite sexes in these forms by the signs (+) and (−) on account of a generally greater vegetative luxuriance of one sex over the other. That

[1] Report of investigation carried on, 1912–13, at the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N. Y.

in reality the two sexes are represented in the (+) and (−) groups is shown by the sexual reaction which may occur not only when the (+) and (−) races of the same species are grown together and perfect zygospores are produced, but also by the sexual reaction which may occur when (+) and (−) races belonging to different species are grown together. This reaction between the opposite races of different species has been called "imperfect hybridization" since it does not lead to the formation of perfect hybrid zygospores, but usually stops short with the formation of progametes, though occasionally gametes are produced which, however, never unite.

A sexual race of a dicœcious species if grown between the (+) and (−) races of another test species used as a standard, will show a line of imperfect hybridization on one side only. Some



Petri dish culture showing dark line of zygospores between the (+) and (−) strains of a species of *Choanephora* (*MC*) and white lines of "imperfect hybridization" between the strains of this species and the opposite strains of *Mucor V.* (*MV*).

of the hermaphroditic species, on the other hand, when similarly grown, show a response to both (+) and (−) test races and produce therefore two lines of imperfect hybridization. When

the sexual reaction is very strong it may become evident to the naked eye by the formation of a distinct white line as is shown in the adjoining photograph.

Some few species in the hermaphroditic group are distinctly heterogamic with a constant difference in size between the conjugating gametes. It seems reasonable to consider the larger gamete female and the smaller male. Upon this basis, if a sexual reaction could be established between these unequal gametes and the (+) and (−) races, the race reacting with the larger female gamete must be considered male, while the race reacting with the smaller male gamete must be considered female. This was pointed out in 1906 ('06*b*) and in a recent article ('13*b*) it has been shown in brief that sexual reactions have been induced between unisexual races and heterogamic species. The conclusion is reached that the (−) race is to be considered male and the (+) female. The present article will give the detailed results which have formed the basis for this conclusion.

Heterogamic Hermaphrodites.

Since inequality in the size of the gametes in heterogamic species is used as the criterion of sex, it is essential that there be no doubt as to the process of sexual reproduction in these forms. With the exception of *Syncephalis*, which is difficult to cultivate, the heterogamic forms at present known are *Dicranophora fulva* and an undescribed American Dicranophora, neither of which are now in cultivation, *Absidia spinosa*, *Zygorhynchus heterogamus*, *Z. Moelleri* and a number of forms recently described (including *Z. Vuillemini*) which are perhaps too closely allied to *Z. Moelleri* to be deserving of separate names.

The writer in a recent publication ('13*a*) has made a restudy of the genus *Zygorhynchus* and attempted to correct certain misinterpretations of zygospore formation which had been advanced for this genus. Moreau ('12, '13) also, both before and since the publication mentioned, has insisted upon the correct interpretation. So far as concerns the formation and union of unequal gametes, the process is essentially similar to that in *Absidia spinosa* which is the heterogamic species most extensively used in the tests discussed in the present paper.

Absidia spinosa was first found by Lendner and is correctly described by him (Lendner '08). Its zygospores agree with other Absidias in possessing circinate outgrowths which develop from the suspensor soon after the formation of gametes. It differs from other Absidias, which it resembles in its method of nonsexual multiplication, by being hermaphroditic (cf. right-hand side of Fig. 8, Plate I.) and heterogamic (Fig. 4). Figs. 1–7 represent stages in development in living material drawn at the times indicated in the legend. A considerable number of conjugations have been followed in living material and stages have been studied in stained and mounted material. Unequal gametes, such as are shown in Fig. 4, unite, as in Fig. 6, and the zygote thus formed grows into a mature zygospore such as is shown in Fig. 7. In moist chamber cultures, drops of fluid generally accumulate around the uniting gametes (Fig. 6), often causing appearance of transverse lines that suggest additional cross walls. They can be distinguished from cross walls, however, by forcing the conjugating filaments against condensed moisture on the under side of the cover-glass. Perhaps the most typical form of conjugation is that shown in Fig. 8, where a rather stout branch on the right has applied itself to the more delicate termination of the main axis causing the production from the latter of a small male gamete, while it produces from its own enlarged tip a large female gamete. Curved outgrowths arise from the swollen suspensor but are not produced from the side of the smaller gamete. In *Zygorhynchus* a septum is regularly formed across the main axis just above the origin of the stout conjugative branch. This septum is as regularly absent in *Absidia spinosa*.

When for any reason the process of conjugation is arrested after the formation but before the union of gametes, these sexual cells may round themselves off, form thick walls and become azygospores. This is brought about in greater or less degree when the conjugating filaments in a moist chamber culture become immersed in a drop of condensed water on the underside of the cover-glass. Whatever the cause of the check to normal conjugation, the size of the azygospores depends upon the size of the gametes from which they develop. Thus in Fig. 33 it

would be possible to label the azygospores of *A. spinosa* male and female, from their relative size alone, even if one were not subtended by a larger suspensor and surrounded by the outgrowths peculiar to the side of the female gamete. Similarly *Zygorhynchus* may produce male and female azygospores from adjacent gametes that have failed to fuse (Fig. 13). As will be shown later, azygospores may be produced from gametes that have formed as the result of a sexual reaction with a different species. If the opposite gametes of hermaphroditic forms are strictly male and female, it would theoretically be possible to transform a hermaphroditic species into male and female races by germinating its male and female azygospores. This has been attempted ('06*a*) and germinations have been obtained of the azygospores of *Sporodinia*. The mycelia that have thus been obtained produced only a very scanty growth and soon died out before it was possible to test their sexual reaction. Germinations of other azygospores, so far as the writer is aware, have never been critically investigated.

Homogamic Hermaphrodites.

Spinellus fusiger, *Sporodinia grandis*—two forms found upon fleshy fungi,—*Mortierella polycephala* and *Mucor Genevensis*—are the only homogamic forms definitely known among hermaphroditic Mucors. In none of these is there apparently a constant difference in the size of the gametes. Neither *Spinellus* (which is difficult to cultivate on artificial media) nor *Sporodinia* shows sexual reactions with other species. *Mortierella* has not been available for study. *M. Genevensis*, of which the writer has races from four distinct sources as well as derived mutants, shows a strong sexual reaction with both (+) and (−) sexes of diœcious species. The reaction has already been noted ('04*b*, p. 311, pl. IV., fig. 56). Observation on living material shows the reaction to be similar to imperfect hybridization between different diœcious species. The formation of azygospores, however, has been observed in different races of this hemaphroditic species as a result of the stimulus of contact with (+) and (−) strains of Mucor V. Azygospore formation will be further discussed under *Absidia spinosa* below.

Diœcious Species.

No diœcious form of the Mucors is known to be regularly heterogamic. In *Rhizopus* ('04*b*, Fig. 15) and in other diœcious species the writer has found that the inequality in size between conjugating gametes has no necessary relation to sex, since the larger gamete is formed sometimes by one sex, and sometimes by the other. In many species, however, the (+) race regularly shows a decidedly greater vegetative vigor than its (−) mate. This greater vigor of growth may be responsible for the fact that the conjugative filaments arising from the (+) mycelium average stouter than those from the (−), and this difference may be accompanied by a similar difference in the gametes and suspensors. One was generally able to recognize the (+) from the (−) sides of the line between the two sexes of Mucor V. by this greater vigor of the (+) conjugative filaments but even in this form where the distinction is most marked, the stouter of the conjugating filaments is not invariably on the (+) side. Mucor V. is a form found by the writer ('04*b*) in 1904. It is apparently not specifically distinct from *M. hiemalis* Wehmer, the sexual races of which were isolated by Hagem ('08) and forms zygospores, though in no great abundance, with the strains of this name sent from the "Centralstelle" at Amsterdam. It differed from the Amsterdam material, however, in its much greater sexual vigor. In 1912 when the reactions described in the present paper were investigated, the Amsterdam *M. hiemalis* failed to show any reactions with other species tested, while Mucor V. was sexually the most active of all the Mucors known. At the present writing, however, Mucor V. has apparently become weakened in sexual activity. Hagem ('08) reports a similar loss of sexual activity in one of his strains of *M. hiemalis*.

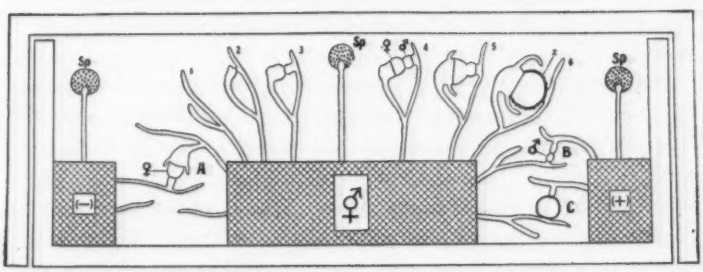
Reactions between Diœcious Species and Absidia spinosa.

The difficulties in technique involved in following the sexual reactions in a thicket of filaments have been overcome by growing the heterogamic hermaphrodite (☿) in a Petri dish between the (+) and (−) test strains and cutting channels in the nutrient agar between the different growths. If the Petri dish be

then inverted, the growth of the reacting filaments may be followed in mid air in the channels, under the low power objective.

Absidia spinosa is the only heterogamic species which has been found to give reactions with both (+) and (−) races and Mucor V. is the only diœcious form that will react with both male and female gametes of *A. spinosa*. These have accordingly been most extensively employed in making the tests shown in the accompanying diagram.

In the left-hand channel at *A* in the diagram, a filament from the (−) race is shown giving a sexual reaction with the larger ♀ gamete of the hermaphrodite, while in the right-hand channel at *B* a filament of the (+) race is figured, showing a sexual reaction with the smaller ♂ gamete of the hermaphrodite. The



Diagrammatic representation of a Petri dish culture showing a heterogamic hermaphroditic mucor (☿) in the center separated by channels on either side from the (+) and (−) races, respectively, of a diœcious species.

Sp., Sporangia containing spores by means of which the plant may be reproduced nonsexually.

1–6, stages in development of a hermaphroditic zygospore from unequal male and female gametes.

A, sexual reaction between a (−) filament and female gamete.

B, sexual reaction between a (+) filament and male gamete.

C, a male azygospore formed at stimulus of contact with a (+) filament.

male gamete, which has been cut off from a filament of the hermaphrodite at the stimulus of contact with a (+) hypha, frequently surrounds itself with a thick wall and assumes the appearance of a resting azygospore, as is shown at *C*.

Figs. 20–24, Plate II., are drawings from living material. The female gamete of *A. spinosa* is recognized not only by its

larger size but also by the curved outgrowths that arise from its suspensor. It will be observed further from these figures that the female gamete is formed from a rather stout branch which appears to have been attracted by Mucor V. (−) and drawn away from the delicate termination of the main axis with which it would normally have conjugated (cf. Fig. 8). In order to obtain such reactions as shown in Figs. 20–24, some care had to be exercised in regard to the condition of the culture. If the nutrient is too favorable for growth the filaments will choke the channels and cannot be followed. If the nutrient is deficient, conjugations will not occur. The depth and width of the channels and the time at which they are cut must be carefully regulated or no reactions can be observed. They have been obtained and studied in a sufficient number of cases, however, to leave no doubt as to their occurrence. Mucor V. is the only form whose (−) race was found to give a sexual reaction with *A. spinosa*.

The sexual reaction of *A. spinosa* with the (+) race of Mucor V. is entirely different from what has just been described. At the line of contact between the two mycelia, the filaments are much branched and imperfect hybridizations are abundant. Figs. 18 and 19 show simplifications of this condition from living material. Fig. 17 shows gametes cut off from *A. spinosa*, while Figs. 14–16 show such gametes transformed into dark thick-walled azygospores. Fig. 15, which shows the progamete of Mucor V. (+) rounded off and in bare contact with the azygospore of *A. spinosa*, is a typical condition.

The (+) race of *Absidia cærulea* (*A* (+), Figs. 8–10) shows a less active sexual reaction with *A. spinosa* but similar to that shown by Mucor V. (+), and the same is true of *A. cylindrospora* and Mucor N. The (+) strains of all four diœcious species mentioned are capable of stimulating the production of small male gametes from filaments of *A. spinosa* and their transformance into dark thick-walled azygospores. Unfortunately it has not yet been possible to bring these azygospores to germination. It would be interesting to discover if their germinations would give rise to unisexual male mycelia.

Judging from the reactions just described between *Absidia*

spinosa and the sexual races of Mucor V., one would seem justified in considering the vegetatively more vigorous (+) race as female, and the less vigorous (−) race as male. The terms male and female therefore may be used hereafter in distinguishing the sexual races of dicœcious Mucors.

REACTIONS BETWEEN DICŒCIOUS SPECIES AND ZYGORHYNCHUS.

Zygorhynchus Moelleri shows no sexual reaction with the (−) race of Mucor V. but contact with its (+) race causes the production of delicate, contorted, warty filaments which take part in imperfect hybridizations as shown in Fig. 12. The gametes formed by *Z. Moelleri* in contact with Mucor V. (+), frequently are transformed into azygospores shown in Fig. 11. *Z. Vuillemini* apparently is identical with *Z. Moelleri*, at least so far as its sexual reactions with Mucor V. are concerned.

In contrast with these two Zygorhynchus forms just discussed which show only a (−) or male tendency in contact with Mucor V., *Z. heterogamus* gives evidence of a female tendency. Its reactions with the (−) race of Mucor V. are shown in Figs. 25–28. It does not seem to react with (+) races and even with the (−) race it is not easy to induce reactions and those that result do not lead to azygospore formation. If Mucor V. (−) in Fig. 25 is actually showing a sexual reaction with the male gamete of *Z. heterogamus* as might appear from its position on the terminal filament of the latter, the evidence would be in conflict with that to be drawn from the reactions with *A. spinosa*, *i. e.*, that the (−) race is male and the (+) race female. It will not be possible to attach much significance to the individual reactions in *Zygorhynchus*, however, since so far no form of this genus has been induced to react with more than a single sexual strain of the test dicœcious species.

Z. Vuillemini var. *agamus* is a zygosporeless strain which Namyslowski (10) separated from a culture of *Z. Vuillemini*. It was found impossible to induce zygospore formation from cultures of this species supplied by the Centralstelle. In contact with the (+) race of Mucor V. (Figs. 30–32) however, it reacted like the sexually active strain of *Z. Vuillemini* and even produced azygospores (Fig. 32). This form therefore has a male tendency.

Judged by their reaction with the (+) and (−) strains of Mucor V., the forms *Z. Moelleri*, *Z. Vuillemini* and *Z. Vuillemini agamus* have a male tendency, while *Z. heterogamus* has a female tendency. It is in harmony with this classification that *Z. Vuillemini agamus* produces azygospores in contact with *Z. heterogamus* (Fig. 29).

Summary

It has been shown that sexual reactions called "imperfect hybridization" may occur when hermaphroditic species of Mucors are grown in contact with the sexual races of dicœcious forms. Certain of these hermaphrodites are heterogamic, showing a constant difference in the size of their gametes. It is assumed that the larger gamete is female and the smaller one male. The race of dicœcious Mucors provisionally called (+) shows a sexual reaction with the smaller or male gamete while the (−) or vegetatively less vigorous race shows a reaction with the larger or female gamete. It is concluded therefore that the (+) race of dicœcious Mocurs is female and the (−) race, male.

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EXPLANATION OF PLATES.

All figures were outlined with the aid of a camera lucida. Those in outline were taken from stained and mounted material. Those stippled show development in living material from moist chamber cultures. Figs. 8–12, 18–24 and 31–32 were viewed with a low power objective and were magnified by the use of increased tube length or higher eye-pieces. Other figures represent high power drawings.

PLATE I.

FIGS. 1–7. *Absidia spinosa.* Consecutive stages in zygospore development from living material. Only those outgrowths in median focus are represented. Drawings were made at following hours: Fig. 1 at 4.15 P.M., Dec. 4; Fig. 2 at 4.52 P.M.; Fig. 3 at 5.50 P.M.; Fig. 4 at 7.10 P.M.; Fig. 5 at 12.30 A.M., Dec. 5; Fig. 6 at 2.15 A.M.; Fig. 7 at 3.00 P.M. The dotted circle in Fig. 6 represents the outline of a drop of fluid around the young zygote.

FIGS. 8–10. "Imperfect hybridization" between *Absidia spinosa* (♀) and the (+) race of *Absidia cærulea* (*A*(+)). Fig. 8 on right, typical conjugation of *Absidia spinosa;* on left, "imperfect hybridization." Fig. 9 drawn at 8.30 A.M., Sept. 23. Fig. 10 drawn at 4.00 P.M., Sept. 24. Note azygospore formed from the male gamete of the ♀ hypha.

FIGS. 11 AND 12. "Imperfect hybridization" between *Zygorhynchus Moelleri* (♀) and *Mucor V.* (+). Fig. 11, male azygospore from ♀ hypha. Fig. 12, three "imperfect hybridizations."

FIG. 13. Azygospores formed from male and female gametes of *Zygorhynchus.*

FIGS. 14–17. "Imperfect hybridizations" between *Mucor V.* (+) and *A. spinosa* (♀). Figs. 14–16, azygospores developed from male gametes of the ♀. Fig. 17, gametes on the ♀.

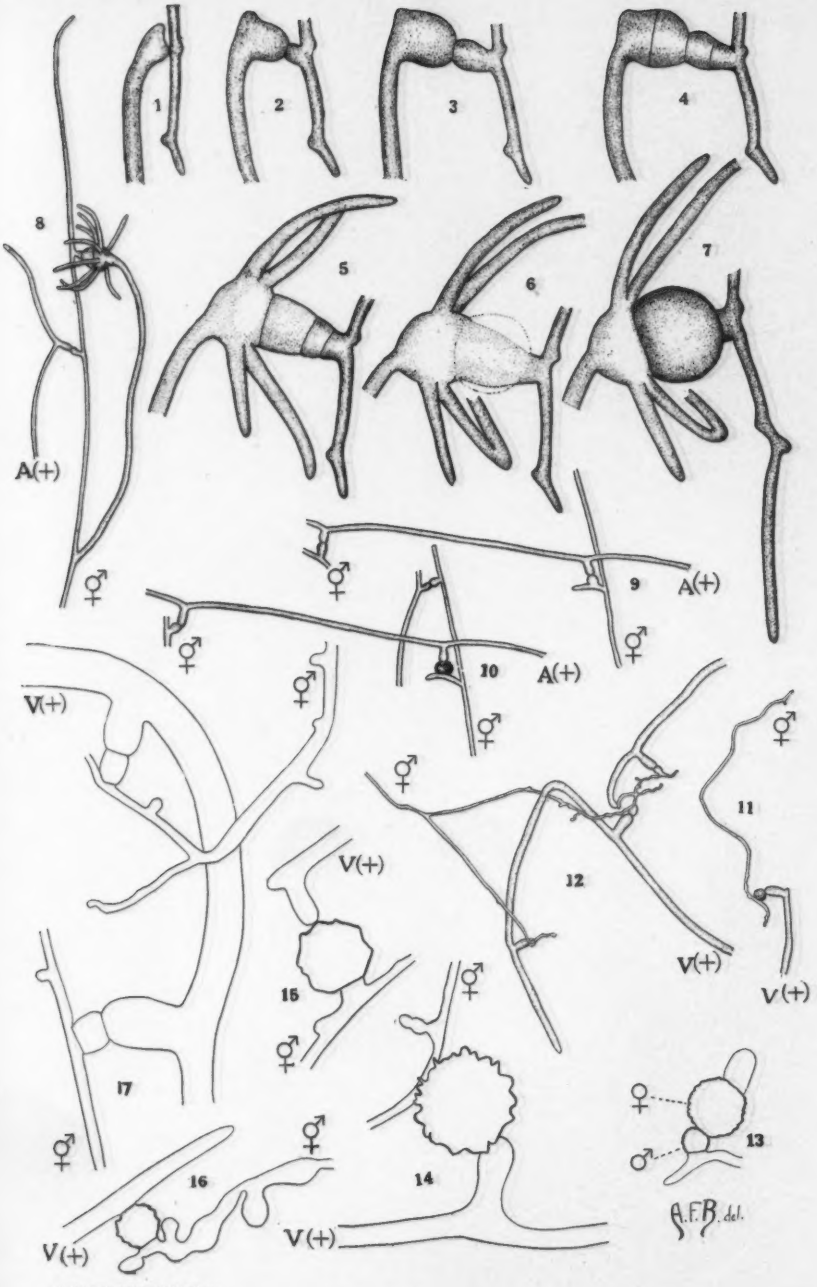

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PLATE II.

FIGS. 18 AND 19. "Imperfect hybridizations" between *Mucor V.* (+) and *A. spinosa* (♀) drawn from living material.

FIGS. 20–24. "Imperfect hybridizations" between *Mucor V.* (−) and *A. spinosa* (♀). Fig. 20 drawn at 7.30 A.M. Fig. 21, the same at 3.30 P.M., Sept. 12. Fig. 23 drawn P.M., Sept. 12. Fig. 24, the same, A.M., Sept. 13.

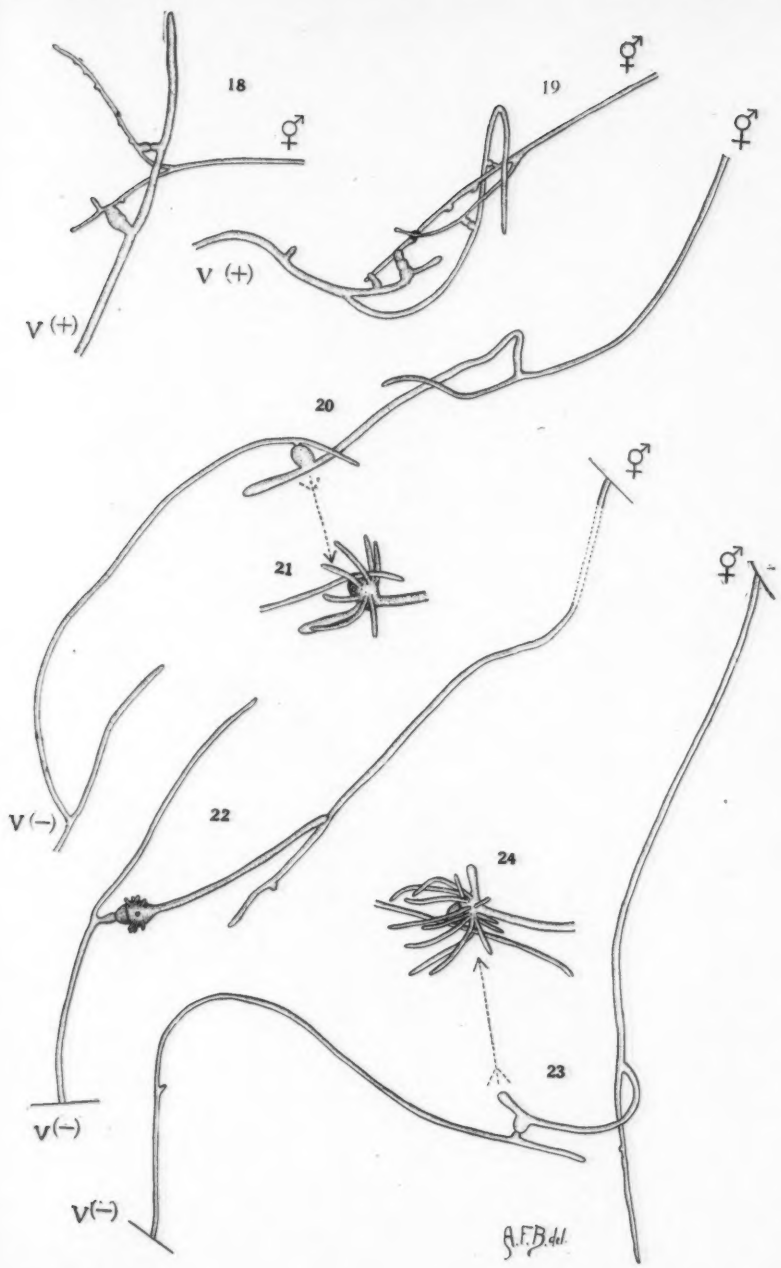

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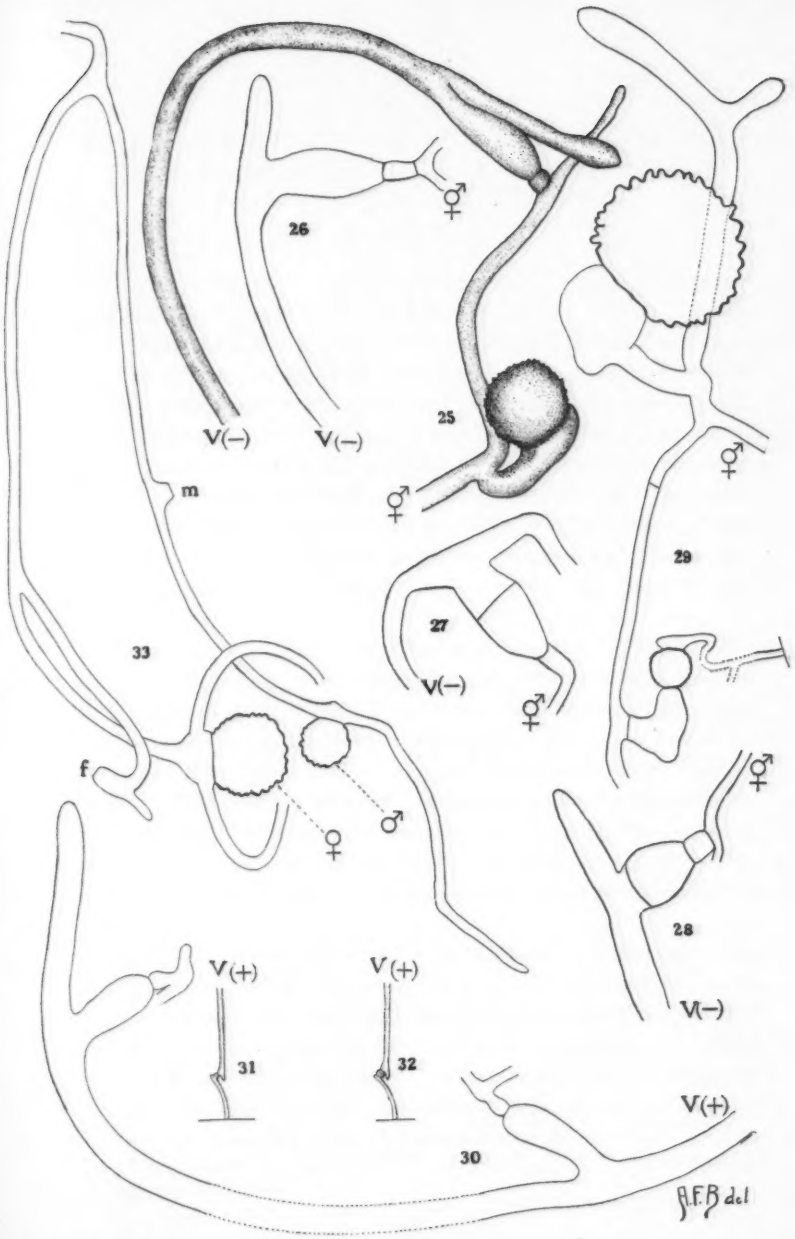
PLATE III.

FIGS. 25–28. "Imperfect hybridizations" between *Mucor V.* (−) and *Zygorhynchus heterogamus* (♀). Fig. 25 from living material. Figs. 26–28, gamete formation.

FIG. 29. "Imperfect hybridization" between *Zygorhynchus Vuillemini* var. *agamus* and *Zygorhynchus heterogamus* showing formation of azygospore from gamete of former species.

FIGS. 30–32. "Imperfect hybridizations" between *Mucor V.* (+) and *Zygorhynchus Vuillemini* var. *agamus*. Fig. 30, progamete formation. Fig. 31 drawn at 9.55 P.M., Dec. 20. Fig. 32 drawn at 10.45 A.M., Dec. 21.

FIG. 33. Apposed azygospores of *Absidia spinosa* developed from male and female gametes. *m* and *f* apparently represent respectively male and female progametes which have been pulled apart in mounting.



ALBERT FRANCIS BLAKESLEE.

REVERSIBLE ACTIVATION AND INCOMPLETE MEMBRANE FORMATION OF THE UNFERTILIZED EGGS OF THE SEA URCHIN.[1]

JACQUES LOEB.

1. The writer showed in 1913 that the artificial activation of the egg can be reversed under certain conditions.[2] When the egg of *Arbacia* is treated with a base in the proper concentration and for the proper length of time the effect is somewhat similar to that which follows the treatment of the egg with a fatty acid. The egg is induced to develop, it may segment (as a rule abnormally) once or twice, and if nothing else happens it will perish rather rapidly (unless the developmental processes are prevented). If the eggs after the treatment with the acid or with the base receive a treatment with a hypertonic solution of the proper concentration and for the proper time they may develop into larvæ.

The writer found that if the eggs are treated with alkali in the proper way to induce development and if they are immediately afterwards put for several hours into a solution which prevents their development (sea-water with chloral hydrate or NaCN) the eggs when taken out behave as if nothing had been done to them. They neither segment nor do they disintegrate and they can again be induced to develop by fertilization (or probably with the methods of artificial parthenogenesis though the writer has not yet tried this). The activating effect of the alkali is therefore reversible.

This reversibility is, however, only possible if the eggs have not been exposed to the alkali too long. After too long an exposure the effect of the alkali is no longer reversible by a temporary suppression of the developmental processes. A second condition for the reversibility is that the eggs are immediately transferred from the alkaline solution into the chloral hydrate

[1] From the Rockefeller Institute for Medical Research, New York.

[2] Loeb, *Science*, N. S., XXXVIII., 749, 1913; *Arch. f. Entwcklngsmech.*, XXXVIII., 277, 1914.

or NaCN solution. If they are first transferred into normal sea-water and then after fifteen or thirty minutes later are transferred to the cyanide solution nothing may happen to them as long as they remain in the cyanide solution (unless the HCN evaporates) but they will disintegrate when put back into normal sea-water. From this we must conclude that in the sea-water the processes started in the alkaline solution will continue and the egg behaves as if it had been overexposed to the hyperalkaline solution.

Somewhat similar experiments were made with the eggs of *Arbacia* which had been treated with butyric acid. When such eggs were put over night into the cyanide-sea-water immediately after the artificial membrane formation they did not as a rule disintegrate when put back into normal sea-water but could be fertilized afterwards. The writer is, however, not certain that the phenomenon of reversibility is as constant here as in the case of the alkali treatment.

The writer pointed out that the explanation for this phenomenon could probably be found if we compare the behavior of the eggs of *Arbacia* with that of eggs of *purpuratus* under similar conditions. If we cause the production of a butyric acid membrane in the egg of *purpuratus* the activation of the egg is usually irreversible. In the egg of *purpuratus* the membrane which is formed under the butyric acid treatment is very tough and is separated by a wide area from the protoplasm, while in the egg of *Arbacia* the membrane consists often only of a fine gelatinous film which lies tightly around the egg. The writer concluded from this that there may be a quantitative if not a qualitative difference in the amount of change produced by the butyric acid treatment in the cortical layer of the two kinds of eggs; in the egg of *Arbacia* where this change is quantitatively smaller the activation is reversible, while in the egg of *purpuratus* where the change is quantitatively larger (and possibly also qualitatively different) it is irreversible (Loeb, "Artificial Parthenogenesis and Fertilization," Chicago, 1913, p. 286).

2. This idea is further supported by the curious phenomena of reversibility in the eggs of *Strongylocentrotus purpuratus*. When one tries to induce artificial parthenogenesis in the eggs

of *purpuratus* by a treatment with a hypertonic solution (without artificial membrane formation) it is found that this is possible only with the eggs of certain females. Such eggs develop into plutei. In the eggs of other females a small percentage of eggs will begin to segment and they may go to the 2, 4, 8, or 16-cell stage or still a little further but then stop developing. Such eggs will go into the resting stage again and are normal to all external appearances. If fertilized with sperm a number of hours or a day later the individual blastomeres will each form a special fertilization membrane and develop into small but apparently perfect larvæ.[1] This experiment shows that the activating effect which the hypertonic solution had was reversed, since all these eggs were at first in the state of active development.[2]

3. The writer wishes to report in this paper the fact that in the egg of *purpuratus* under definite conditions a membrane formation can be produced by butyric acid which leads neither to any development if followed by the usual treatment with a hypertonic solution nor to a rapid disintegration when not followed by any "corrective" treatment. Since in all previous observations on the effects of membrane formation by butyric acid the reverse was found it seemed of theoretical importance to study this exception.

The main condition for the experiment is that the eggs are put after the treatment with butyric acid into a $m/2$ solution of $NaCl + KCl + CaCl_2$ instead of into sea-water; the various salts are always used in the proportion in which they are contained in sea-water. The eggs were first washed three times in a mixture of $m/2$ $NaCl + KCl + CaCl_2$ and were then put for from one and a half to two and a half minutes into a mixture of 50 c.c. $m/2$ $NaCl + KCl + CaCl_2$ + 1 c.c. $N/10$ butyric acid; from here they were transferred into a neutral or a slightly alkaline solution of $m/2$ $NaCl + KCl + CaCl_2$. In this solution all

[1] Loeb, *Arch. f. Entwcklngsmech.*, XXIII., 479, 1907; "Artificial Parthenogenesis and Fertilization," Chicago, 1913, p. 237.

[2] Only the activating effect of the hypertonic solution is reversible, the second, corrective effect, which the hypertonic solution imparts to the egg was not reversed since these blastomeres develop also if only the artificial membrane formation is induced in them by butyric acid, while without the previous treatment with a hypertonic solution such eggs would soon perish. Loeb, *Jour. Exper. Zool.*, XV., 201, 1913; "Artificial Parthenogenesis and Fertilization," p. 238.

the eggs form a membrane which is very thin and which is at first at a great distance from the egg. Figs. 1 and 2 give an idea of the first appearance of this membrane; the membrane in Fig. 1 was formed in a neutral, that of Fig. 2 in a faintly alkaline solution. Later on, the membrane collapses and is found lying rather close to the egg. Such eggs can be readily fertilized with sperm in spite of the existence of the membrane. The latter is either naturally permeable for sperm or it is easily torn and thus allows the sperm to reach the egg or it is not entirely continuous around the eggs.

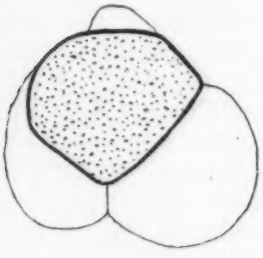


FIG. 1.

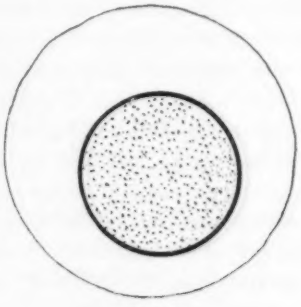


FIG. 2.

Eggs were washed three times in a mixture of $m/2$ NaCl + $CaCl_2$ + KCl and then put for from one and a half to two and a half minutes into 50 c.c. $m/2$ NaCl + KCl + $CaCl_2$ + 1.0 c.c. $N/10$ butyric acid. From this solution they were transferred:

(*a*) Into normal sea-water.[1]

(*b*) Into a neutral mixture of $m/2$ NaCl + KCl + $CaCl_2$.

Lot *a* formed normal fertilization membranes and disintegrated in a few hours. The eggs of lot *b* formed the fine fertilization membrane which was at first very distant from the egg. Some of these eggs were transferred into normal sea-water after one hour. These eggs did not form a better membrane but perished in the same way as the eggs of lot *a*.

A second portion of lot *b* was transferred into normal sea-water after having been for seven hours in the mixture of $m/2$

[1] Such eggs have at first a tendency to stick to the glass when transferred to sea-water. It is necessary to keep them in slight agitation for some time until they have lost their tendency to stick.

NaCl + KCl + $CaCl_2$. These eggs did not disintegrate during the following night although they did not appear quite normal the next day. The rest of the eggs remained in the *m*/2 NaCl + KCl + $CaCl_2$ solution for twenty-four hours. When these eggs were put into sea-water they did not disintegrate. To some of these eggs sperm was added the next morning and about 20 per cent. developed.

This experiment indicates that the nature of the membrane and the cortical changes which activate the egg of *purpuratus* are to a large extent determined by the solution into which the eggs are put after the butyric acid treatment. If the eggs are put directly into sea-water after the butyric acid treatment they form the typical membrane and rapidly perish if nothing else happens to them. The same may be the case if they are transferred from the butyric acid solution into a neutral solution of *m*/2 NaCl + KCl + $CaCl_2$ for a short time (less than one hour) and if they are afterwards transferred into normal sea-water. But if they remain in the NaCl + KCl + $CaCl_2$ solution for a number of hours they will as a rule not disintegrate if put back into sea-water.

A second experiment may be quoted. Eggs (that had been washed three times in a neutral *m*/2 NaCl + KCl + $CaCl_2$ solution) were put for one and a half to two and a half minutes at a temperature of about 14° into 50 c.c. of the same solution + 1 c.c. *N*/10 butyric acid. From here the majority of the eggs were transferred into a neutral solution of *m*/2 NaCl + KCl $CaCl_2$ and the rest into sea-water. The latter formed normal fertilization membranes and disintegrated the same day. Those transferred into the neutral NaCl + KCl + $CaCl_2$ solution had all formed membranes which were at first separated from the egg by a great distance. They were kept in this solution over night and the next morning they were transferred into normal sea-water. All looked normal except that the membrane now formed a fine veil lying closely to the egg. Some of these eggs were fertilized with sperm. They all segmented and developed into swimming larvæ. The others were kept in sea-water without sperm to find out whether they would now disintegrate or whether the effect of the butyric acid treat-

ment had worn off. This was the case. The eggs no longer disintegrated in sea-water. A fraction of these eggs was fertilized later and segmented.

When the eggs are transferred into normal sea-water immediately after the butyric acid treatment they perish rapidly if no second treatment is given to them. If they are treated with hypertonic sea-water (50 c.c. sea-water + 8 c.c. 2 1/2 *m* NaCl or NaCl + KCl + $CaCl_2$) for from thirty to sixty minutes they will develop into larvæ. If they are treated longer they will begin to develop but form very abnormal larvæ.

If the eggs, however, after the butyric acid treatment are not put into sea-water but into a mixture of *m*/2 NaCl + KCl + $CaCl_2$ in which they form the thin membrane and if they are kept here until they no longer disintegrate in normal sea-water, they cannot be induced to develop when treated for from thirty to sixty minutes with a hypertonic solution. Such eggs have, therefore, in spite of their membrane formation gone back into the condition of a resting egg.

4. It was obvious from these experiments that the constituents of the sea-water determine the nature of the membrane after butyric acid treatment. The writer expected to find that Ca was an absolute prerequisite for the membrane formation but this was not the case, although the presence of $CaCl_2$ improves the character of the membrane. Eggs that had been taken out of a *m*/2 NaCl solution were treated with 50 c.c. *m*/2 NaCl + 1 c.c. *N*/10 butyric acid for one and a half to two and a half minutes. They were then transferred into the following three sets of solutions:

(1). NaCl + KCl,
(2). NaCl + $CaCl_2$,
(3). NaCl + KCl + $CaCl_2$,

which were made in duplicates, the one set remaining neutral, the second set faintly alkaline (0.5 c.c. *N*/100 NaOH were added to 100 c.c. of the solution). The result was not very different in the neutral and alkaline solutions. Many but not all the eggs put into NaCl + KCl formed very thin membranes often only in part of the egg. The membrane formation does therefore not require the addition of $CaCl_2$, but the eggs transferred

into NaCl + $CaCl_2$ or into NaCl + KCl + $CaCl_2$ had formed a better and apparently stronger membrane than those put into NaCl + KCl.

In another experiment it was found that in a mixture of NaCl + $MgCl_2$ in the proportions in which these two salts occur in the sea-water a membrane formation was also possible, but the membrane had the same fine and thin character. In all these cases the eggs could be fertilized with sperm in spite of the membranes. The writer is under the impression that no second membrane was formed upon the addition of sperm and only the hyaline membrane developed later. That the eggs after this inefficient membrane formation were not in the same condition as eggs which are put into normal sea-water after artificial membrane formation was shown by the fact that when put into a hypertonic solution for from thirty to sixty minutes not a single egg could be induced to segment.

The fact that the membrane always collapsed after some time seems to indicate that it soon became permeable for the colloids liquefied at the surface of the egg in the process of membrane formation.

5. The explanation of the phenomenon of ineffective membrane formation will probably be the same as that for the cases of reversible activation of the egg. It is an established fact that the rate of oxidations in the egg of *purpuratus* is raised about 600 per cent. by the act of fertilization. This rise is entirely due to the alteration of the surface layer of the egg which results in the membrane formation since artificial membrane formation alone has the same effect on oxidation. Even if the egg is killed in the process of membrane formation, *e. g.*, if we treat the unfertilized egg with saponin and leave it in this solution, the rate of oxidation is raised to the same amount.[1] The amount to which the artificial membrane formation raises the rate of oxidations in a sea-urchin egg seems to be a constant for each species and not to depend upon the nature of the agencies employed for this purpose.

When we treat the unfertilized eggs of *purpuratus* with a hypertonic solution (without inducing a membrane formation) the

[1] Loeb and Wasteneys, *Jour. Biol. Chem.*, XIV., 479, 1913.

eggs of some females will develop into larvæ, while the eggs of others will not. The writer assumed that the hypertonic solution if applied to the unfertilized egg has two effects, one consisting in the alteration of the surface layer (comparable to the effect of butyric acid) and secondly the corrective effect. Wasteneys and the writer[1] published a series of measurements on the influence of a hypertonic solution on the rate of oxidations in unfertilized eggs of *purpuratus*, and found that the effect varied enormously with the eggs of various females. The eggs were in the hypertonic solution for one hour and the rate of increase in oxidation varied for the eggs of various females between 40 and 400 per cent. As we stated above, the eggs of only a limited number of *purpuratus* can be induced to develop into larvæ by a mere treatment with a hypertonic solution and it is probable that the eggs in which the oxidations were raised efficiently were the ones that could be induced to develop into larvæ in this way, while those in which the rate of oxidations was not raised to the same height did not segment. We also noticed that in the latter type of eggs the rate of oxidations diminishes after some time. It is possible that the reversion of development in such cases is due to a decline in the rate of oxidations below the height required for development.

The same possibility holds for the lack of development of the eggs after artificial membrane formation through butyric acid when they are afterwards put into a solution of NaCl + KCl + $CaCl_2$ (instead of into sea-water). The fact that no correct membrane is formed and that the eggs neither develop nor disintegrate, and behave towards a treatment with the hypertonic solution like eggs without a membrane, arouses the suspicion that in this case the butyric acid treatment did not lead to the proper increase in the rate of oxidations. It is intended to investigate this possibility next summer, since it may also furnish the explanation of the phenomena of reversibility in development.

[1] Loeb and Wasteneys, *Jour. Biol. Chem.*, XIV., 474, 1913.

REVERSIBILITY OF THE REACTIONS OF PLANARIA DOROTOCEPHALA TO A CURRENT OF WATER.

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I. INTRODUCTION.

A reaction of planarians to currents of water has been described by Pearl ('03) in his careful study of the general features of the behavior of planarians. He has described the reaction and his method of obtaining it as follows: "In the course of the experiments to localize chemical stimuli by the capillary tube method, it was discovered that by means of a tube with a relatively large opening (from .25 to .50 mm. in diameter) and letting the ordinary tap-water in which the animals were flow out of it by its own weight, a current of just the right intensity to cause a positive reaction could be produced. The animals would turn very sharply toward the source of such a current, the reaction being evidently the same as that to other weak stimuli (chemical and mechanical). This reaction is localized in the same way as the usual positive reaction. It is given only when the current is directed against the head or anterior part of the body" (p. 698). He states that earlier in his work a large number of experiments were performed with various devices to determine whether these animals would show such a reaction, but without success. Streams of water from a

pipette and similar devices caused only a stopping, longitudinal contraction, and gripping of the bottom without any turning either toward or away from the source of the stimulus.

That the rheotropic reactions of planarians were found so difficult to demonstrate, it seems probable, must have been due to the experimental methods employed. Using the methods described below, it has been found that rheotropic reactions of these animals can be demonstrated very easily, not only in a "current of just the right intensity" but in currents of a large range of intensities. Under the conditions of these methods a worm is entirely surrounded by the flowing water on all surfaces except the ventral surface which is attached to the substratum, and the conditions of stimulation are more typically rheotropic than when the stream of water is directed as a small jet against a localized part of the body. A negative reaction, *i. e.*, a turning away from the side stimulated, was not described by Pearl but Dr. C. M. Child, who suggested this study, has observed a negative reaction as well as a positive reaction in currents of water in his laboratory stocks of worms used in studies in regeneration. It has been found that these reactions are reversible experimentally. The study of their reversibility which is reported in the present paper was preliminary to a more detailed study of the rheotropism of these animals which is in progress at the present time.

The work reported in the present paper was done some time ago in the zoölogical laboratory of the University of Chicago. My acknowledgments are due Dr. C. M. Child and Dr. V. E. Shelford for helpful suggestions.

II. Material and Methods.

Planaria dorotocephala has been used exclusively for this study. Specimens were collected from a spring-fed marsh at the margin of the Fox River near Chicago, Illinois. These animals are very easy to keep in the laboratory without special care. Since this study was in the nature of a testing of the reaction possibilities of the worms toward currents of water, rather than an effort to determine the normal habits, no efforts were made to duplicate the normal conditions of existence in

nature. Specimens were kept in large numbers in glass and galvanized iron containers which were emptied of water, rinsed and filled with fresh tap-water from time to time without removing the worms which cling to the surface of the vessel. They were fed two or three times a week with fresh beef cut into small bits and left in the dish for several hours or during the night. These worms collect on fresh meat and secure blood and juices but it is very improbable that they are able to make use of the solid portions.

Rheotropic reactions were tested in a "circular current" as follows: A considerable number of specimens, sometimes several hundred, were placed in a circular shaped vessel such as a glass battery jar or a kitchen pan and the water was stirred vigorously around the dish several times with a stirring rod. Vigorous stirring usually dislodged the most of the specimens which were then swept into a bunch at the center of the current. If they were not dislodged by the stirring, they were loosened by means of more vigorous streams of water from a large bulbed pipette. The movements of the animals on the bottom of the vessel only were studied since those on the sides were in a different relation to gravity which introduced a geotropic factor into the reaction. Rheotropism has been observed, however, in worms gliding on vertical surfaces.

It was found convenient to have all the specimens, or the larger part of them, enter the experiment at the center of the "circular current" since they were then all placed under similar conditions. In whatever direction any worm started out from the center, it would receive the current against the same side of the body as all the other worms starting from the center. If the current were stirred in the clockwise direction, for example, all the worms starting from the center of the dish would receive the current against the left side of the body. If they gave a positive reaction, they turned toward the left side; that is, toward the side stimulated, or up-stream, and if they gave a negative reaction, they turned toward the right side; that is, away from the side stimulated, or down-stream. When a large number of worms were gliding on the bottom of the dish, if their reactions were uniform, a very striking figure was produced.

This is illustrated in Figs. 1 and 2. Fig. 1 is a photograph of a lot of worms in a dish pan, reacting positively to a current stirred in the clockwise direction. The conspicuous spiral form of the figure is characteristic of the positive reaction. Fig. 2 is a photograph showing the characteristic negative reaction near the center of the pan.

These peculiar and characteristic spiral figures in a "circular current" called for a more careful examination of the physical conditions in the experiment, which revealed the fact that the worms were subjected to a system of spiral currents instead of a circular current. If a drop of a water suspension of carmine is placed on the bottom of a dish in which the water has been stirred in this way, the carmine in the lower layers of water close to the bottom will be seen to stream inward along a spiral course toward the center. Fig. 3 is a photograph of a dish pan in which the water was stirred in the clockwise direction and then drops of carmine were placed on the bottom at eleven points around its circumference. The carmine was dragged along by the currents and left streaks on the bottom of the pan which show as spiral lines in the photograph. The worms were subjected to this spiral system of currents. Carmine in the upper layers of water was swept about the dish in a fairly circular direction, and the water which flowed along the spiral lines on the bottom toward the center, rose, as it neared the center, and spread outward above.

A comparison of Fig. 3 with Fig. 1 shows that the spiral lines of the currents and the spiral lines of the positive reaction are alike. This explains, therefore, the spiral character of the reaction. The worms orient themselves to a spiral current. A comparison of Fig. 2 with Fig. 3 shows that in the usual negative reaction the direction taken is away from the side stimulated, but rather diagonally than directly down-stream. In some cases, however, when worms were distributed over the bottom and were not dislodged by the stirring, they were observed to turn inward and follow along the lines of the spiral current in as precise a negative orientation as was often characteristic of the positive reaction.

A mixture of definitely positive and definitely negative re-

actions at the same time, that is, an intermingling of the two systems of spirals, was not common. The reactions varied, however, in uniformity at different times; that is, in the precision of orientation, and in some cases the worms scattered from the center without forming any very definite spiral in either direction. Such "reactions" were designated "indefinite."

III. Difference of Reaction to Currents of Different Velocities.

In studying the reversal of rheotropic reactions induced by changing the chemical and thermal conditions, it was found that it is important to consider the velocity of the current. A change in the velocity of the current, or, in other words, in the strength of the rheotropic stimulus, may itself cause reversal of the sign of the reaction.

Since a current in a small vessel diminishes rapidly in velocity after the stirring is discontinued, the different worms in a single trial are subject to different velocities of current, the first worms to leave the center entering the strongest current and later worms finding successively weaker current. It was frequently observed that the positive reaction was given by the earlier worms; that is, those in the strongest current, and the negative reaction by the later worms; that is, those in the weaker currrent. The proportion of individuals giving each reaction varied in different observations while in some cases no negative reaction was given and in other cases no positive reaction was given.

A positive reaction in stronger current and a negative reaction in weaker current can be seen at the same time in a broad-bottomed pan where the negative spiral may begin about the center before the last individuals belonging to the positive spiral have reached the sides. This is possible because the current is swifter about the outside where the positive reaction is still being given than about the center where the negative reaction is beginning. Fig. 2 shows such a combination of positive spiral about the outside and negative spiral about the center. In the case which is photographed there is considerable irregularity in the positive spiral. In many cases the positive spiral about the outside was as regular as that in Fig. 1.

Table I. shows the total number of trials in which the different types of reaction were given during all the experiments.

TABLE I.

SUMMARY OF ALL REACTIONS OBSERVED (EXCEPT THOSE GIVEN UPON INCREASING THE VELOCITY OF THE CURRENT, FOR WHICH SEE TABLE II).

The sign + indicates a positive spiral, −, a negative spiral and ?, no dominant spiral of either sign. The sign + − indicates positive reaction in stronger current and negative reaction in weaker current; (+) − indicates only a few worms in the positive spiral, and + (−) only a few worms in the negative spiral.

Reactions.	Total Number of Trials of Worms in Normal Conditions Preceding Experiments, and in Normal "Controls" During Experiments.		Total Number of Trials During Experiments, in all Conditions, Excepting the "Controls."		Totals.	
+	324		119		443	
−	31		130		161	
(+) −	45	113	48	104	93	217
+ −	46		36		82	
+ (−)	22		20		42	
?	32		29		61	
Totals.....	500		382		882	

In the observations described above, the same individuals were not observed to give both reactions because under the conditions of the tests the same individuals are not subjected to both extremes of current velocity. The earlier worms receive the stronger current and the later worms receive weaker current. It was readily shown, however, that the same individuals which responded negatively in a weaker current would respond positively in a stronger current. Upon second stirring of the water over a negative spiral without dislodging the worms, it was generally found that the worms composing the negative spiral reversed their direction of movement from negative to positive, so that the negative spiral became converted into a positive spiral composed of the same individuals. Out of 100 cases in which second stirring of the water over a negative spiral was tried, 80 cases showed reversal to positive. In some cases all the individuals gave this reversal while in other cases few individuals, that is, only those forming the outside of the spiral gave the reversal. In some cases reversal was readily induced by currents of moderate velocity while in other cases reversal was given only in very strong currents.

TABLE II.

REVERSAL OF REACTION INDUCED BY INCREASING THE VELOCITY OF THE CURRENT. SUMMARY OF ALL REACTIONS IN EXPERIMENTS IN WHICH THE VELOCITY OF THE CURRENT WAS INCREASED WHILE A REACTION WAS BEING GIVEN.

Velocity Increased During a Negative Reaction.

Complete Reaction Before Increase in Velocity.	Reactions After Increase in Velocity.				
	Reversal to +.	More Precise —.	No Change —.	Worms Dislodged.	Reaction Became ?.
—	21	5	5	1	1
(+) −	18	1	2		
+	28		3	2	
+ (−)	13				
Totals....	80	6	10	3	1

Velocity Increased During a Positive Reaction.

Complete Reaction Before Increase in Velocity.	Reactions After Increase in Velocity.		
	More Precise +.	No Change +.	
+	8	4	
? +	1		

Velocity Increased During an Indefinite Reaction.

Complete Reaction Before Increase in Velocity.	Reactions After Increase in Velocity.	
	Reaction Became +.	
?	4	
(+) ?	4	
+ ?	15	
+ (?)	1	

No evidence has been obtained that a stronger rheotropic stimulus will cause a negative reaction while a weaker stimulus causes a positive reaction. No observations showed a sequence of reactions during the diminution of the velocity of the current; of negative in stronger current and positive in weaker current (*cf.* Table I.). And second stirring of the current while the worms were distributed over the bottom was never observed to reverse the reaction from positive to negative. If the worms were giving the positive reaction in weaker current they continued to give the same reaction in stronger current, and usually with greater precision of orientation (Table II.). When the reaction was

"indefinite" in weaker current, the stronger current usually called forth the positive reaction.

IV. Reversal of Reaction Induced by Chemical Changes.

Reversal of reaction from positive to negative can usually be induced by pouring off the water from the worms and replacing it with fresh tap-water. Thus in 23 out of 24 observations this treatment resulted in reversal to the negative reaction. Two factors may be responsible for this reversal, change in temperature and change in the chemical composition of the water. Each of these factors was investigated by modifying the condition in question while preserving other conditions unmodified. The velocity of the current could not be preserved unmodified with the methods employed, but this factor was controlled indirectly.

The effect of changing the chemical character of the water was tested as follows: A lot of worms were tested in the water in which they were living at the time. Then an equal volume of fresh tap-water, or modified water, was brought to the same temperature as the aquarium water, and the worms were tested alternately in these two kinds of water.

A number of experiments were performed in which aquarium water was replaced by fresh tap-water of the same temperature. Since the rheotropic reaction in many cases may be positive in stronger current and negative in weaker current, it must be made certain that the reversal of reaction after a chemical change in the environment is a reversal of reaction in the same velocity of current. Although it seemed fairly certain to the operator, during the earlier experiments, that there was such a reversal in the same velocities of current, the later experiments were performed with a "control" to make them more conclusive. In these experiments two dishes of worms living under the same conditions, frequently in the same aquarium tank, were tested side by side. One lot of worms was tested in waters of different composition while the other lot was kept as the "control" and was tested always in the original aquarium water. The experimental and control dishes were placed side by side, and the control worms were tested at each trial simultaneously with

the experimental worms so that the conditions as regards velocity of current were practically the same in the two dishes. Progressive diminutions in velocity were practically the same in the two dishes. Differences of reaction given by the worms in the two dishes at the same time of observation must then be traced to other factors than the velocity of the current.

The following experiment will illustrate the method of the controlled experiments and the characteristic reversal of reaction induced by change from aquarium water to fresh water. Many hundreds of worms of miscellaneous sizes were tested in two large galvanized iron tanks. A current was produced by shaking

TABLE III.

EXPERIMENT SHOWING REVERSAL OF REACTION FROM POSITIVE TO NEGATIVE INDUCED BY CHANGE FROM AQUARIUM TO FRESH WATER AT THE SAME TEMPERATURE.

Trial.	Experiment.		Control.
	Water.	Reactions.	Reactions.
1	Aquarium	+ good general response	+ in strong current − in weak current
2	Fresh	+ on second stirring, even in weak current − even in fairly strong current. − on second stirring, even in fairly strong current, a few + in strongest current	+ on second stirring, universal. + even in very reduced current.
3	Aquarium	− slight; worms slow in distributing on the bottom. + on second stirring, even in only moderately strong current.	+ even in very weak current.
4	Fresh	− even in fairly strong current. − on second stirring, no positive reaction.	+ even in very weak current.
5	Aquarium	movement very slow, current reduced before any reaction was given. + on second stirring only moderately strong current.	+ even in very weak current.

the tanks in the hands, which swept the worms to the center. In the experimental tank aquarium water was used in trials 1, 3 and 5; and this was replaced by fresh water in the alternate

trials 2 and 4. In the control tank the worms remained in the aquarium water in all the trials. The temperature was 21° C. The reactions in each trial are described in Table III.

From the table it can be seen that placing the worms in fresh water reversed the reaction from positive to negative while returning them to their former aquarium water brought back the former positive reaction. These reactions were reversed back and forth by changing the water. The reversal of reaction cannot be attributed to a difference in the velocity of the current. The negative reactions in the fresh water were given in fairly strong current while the positive reactions in aquarium water were given in very weak as well as stronger current; and in the control, in which the velocity of the current was approximately the same at each moment as that in the experiment, a positive reaction was given at the same time that a negative reaction was observed in the experiment. In the two tests in this experiment with fresh water (trials 2 and 4), therefore, a change to fresh water induced a reversal of reaction from positive to negative while a return to the normal environment brought back the positive reaction. In 40 such tests in experiments at different times, a reversal from positive to negative was given in 21 tests and a reversal from negative to positive in 5 tests. In 7 tests a positive reaction remained unchanged while in 5 tests a negative reaction remained unchanged. In 2 tests the reaction was "indefinite."

Fresh water differs from aquarium water in oxygen content and in the absence of the metabolic products of the worms and the decomposition products that accumulate in the aquarium. The aquarium water in experiments at different times varied greatly in composition, while the fresh drawn tap-water probably varied little. It would seem that fresh water would make a more suitable environment than stale aquarium water where many worms have lived together without green vegetation. In the experiment described above the aquarium water was quite foul from the decomposition products of juices of meat that had stood in the aquarium for nearly 24 hours. When modified aquarium water was used instead of fresh water, reversal of the same sort was induced. In one experiment part of

the aquarium water was boiled, and in the two tests made with this boiled aquarium water reversal of reaction from positive to negative was obtained. In another experiment cane sugar was dissolved in part of the aquarium water to make a 1 per cent. solution which induced reversal from positive to negative in the 6 tests made. Similarly a well-mixed solution of juices of raw beef in aquarium water induced the same reversal in the 6 tests made. So far as these experiments indicate, therefore, the reversal of the rheotropic reaction in the new environment seems to be due more to the strangeness of the new conditions than to their fitness or unfitness. In a total of 54 tests with modified aquarium water and fresh water, reversal of reaction from positive to negative was induced in 35 tests, while in 5 tests the reaction was already negative from other causes. These figures may not necessarily represent the proportion of individual worm reactions which can be reversed in this way, since the conditions in different experiments were not entirely identical, but it may be repeated that in each of these tests a large number of worms were tested, in some cases many hundreds of individuals.

V. Reversal of Reaction Induced by Changes of Temperature.

The effect of changes of temperature upon the rheotropism was tested by dividing the water in which worms were living into two portions, preserving one portion at the temperature at which the worms were found at the beginning of the experiment, and cooling or warming the other portion to the desired temperature. The worms were then tested alternately in these two portions of water. It was found that the rheotropism can be reversed from positive to negative by lowering the temperature in the same manner as by changing the composition of the water.

In 34 individual tests, lowering the temperature by 4°–10° C. was found to cause a reversal in 17 tests from positive to negative, and reversal from negative to positive in 2 tests. In 4 tests this change in temperature made an indefinite reaction become positive. In the remaining 11 tests, lowering the temperature produced no observed change in reaction. This remained as before the temperature change; that is, as follows: positive in 7

tests, negative in 3 tests, positive in stronger current and negative in weaker current in one test.

The characteristic rheotropic effect, therefore, of lowering the temperature below that to which the worms are accustomed is a reversal of reaction from positive to negative. Return to the normal temperature brings back the positive reaction so that the reactions can be reversed back and forth by alternately lowering the temperature and raising it again to the normal. The reversal from negative to positive on raising the temperature seems to be due to the return to the temperature to which the the worms are accustomed, rather than to the fact that the temperature is raised, since in 13 tests of raising the temperature above the normal by 4°–10° C. reversal from negative to positive was obtained in only one case. In 4 tests the reversal was in the opposite direction; that is, from positive to negative. In 5 tests the negative reaction remained unchanged and in 2 tests the positive reaction remained unchanged, while in one test the reaction became "indefinite."

VI. Variations in Rheotropic Reactions.

The general features of the rheotropic reactions of planarians and their reversibility described above were observed in mass tests of large numbers of individuals. These depend upon considerable uniformity in the behavior of the different individuals in the trial, much as do the behavior experiments with cultures of protozoa. The uniformity of the reactions of the different individuals in a single trial in the experiments with planarians was generally very striking, at least with respect to the sign of the reaction in the same velocity of current. The precision of the reactions of different individuals varied greatly in different trials, although the sign of the reaction was definite. When the reaction is definitely either positive or negative, the characteristic spiral figure is very striking, and when a spiral could not be seen, the reaction was called indefinite. Only a small number of trials, however, failed to show a general uniformity in the sign of the reaction in the same velocity of current (Table I.).

The different tests of reversal in the same experiment generally gave fairly consistent results although with some variations

among the different tests. But in some experiments the lot of worms gave consistent results directly opposite to those generally obtained. Thus in the experiments of lowering the temperature, five of the seven tests in which the positive reaction remained unchanged were the successive tests in a single experiment. In this case the temperature at the beginning of the experiment, and the amount of the depression of the temperature, were the same as in other experiments in which reversal of reaction was produced in the characteristic manner. Such differences in behavior must be attributed to that complex of internal processes which has been characterized the "physiological state" of the organism.

VII. Reactions to Currents of Water in Nature.

That rheotropic reactions are normal to the life of planarians in nature is shown by field observations. The specimens studied in the laboratory were collected in a spring-fed marsh at the edge of the Fox River near Chicago, called "Station 10 Cary Spring" in Shelford's description of the animal communities of the Chicago Region (Shelford, '13, pages 52, 93 and 118). The marsh bottom is composed of black humus without stones under which the worms could collect in the manner that Pearl found characteristic of this species in the Huron River, but an abundance of leaves serves the same purpose. The water is very shallow and well shaded by marsh vegetation. There is a sluggish current fed by springs at the upper edge of the marsh, where water cress is found growing.

A small channel about five inches wide and fifteen feet long was discovered at the edge of the marsh through which a moderate current of water was flowing. A large number of worms were found in this stream, some moving on the bottom, generally up-stream, but most of them at rest on the under sides of leaves or in sheltered places on the bottom. Removing the shading vegetation for better observation stimulated many of the worms at rest to become active, and half an hour later 60 worms were counted going up-stream and 5 going down-stream and 200 were counted at rest on the under side of leaves. At that time the sun was shining somewhat from the side, and down-stream, but

it was not far from vertical as it was 11:30 in the morning. At 12:20 only 4 worms could be found in sight, 3 going up-stream and one going down-stream. At 1:30, when it was cloudy, 40 worms were found going up-stream and 4 going down-stream. At 3:10 a piece of raw beef was placed in the upper part of the stream and fifteen minutes later 176 worms were counted going up-stream and 34 going down-stream. It has frequently been noted that worms in a moderate stream of water will respond in this way and collect upon a piece of fresh beef and this behavior has been put to practical advantage in collecting specimens. Worms removed from this stream and tested in a circular pan by the method employed in the laboratory showed both the positive and the negative reactions very clearly, the positive in stronger current and the negative in weaker current, as described above.

These observations show that planarians may react to currents of water in nature. Pearl considered it "very improbable that this reaction is of any importance in the normal activity of the animal." This opinion may have been due to the difficulty which he experienced in demonstrating the reaction experimentally which might lead one to think that unusual conditions are necessary for its production in nature; whereas it has been shown that the reaction is given with readiness under experimental conditions which are similar to those in a natural stream of water, and also that it can be observed in nature. It would seem that a reaction which is so characteristic of the animals in the laboratory would play a part in their daily life in nature, when they live in an environment which offers the appropriate stimuli for the reaction. These animals seem to belong characteristically to stream communities where these conditions are fulfilled.

VIII. Summary.

1. *Planaria dorotocephala* show both positive and negative reactions in a stream of water.

2. The sign of the reaction may differ, depending upon the velocity of the current. The positive reaction is then given in stronger current and the negative reaction in weaker current.

3. A negative reaction in weaker current can often be reversed to the positive by increasing the velocity of the current.

4. Reversal of reaction from positive to negative can be induced by changing the composition of the water; and from negative to positive by return to the former conditions.

5. Similar reversals of reaction can be induced by sudden changes in temperature.

6. Rheotropic reactions are given by *Planaria dorotocephala* in nature.

7. Rheotropic reactions are very characteristic of these animals in the laboratory, rather than unusual as has been supposed.

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EXPLANATION OF PLATES.

PLATE I.

FIG. 1. The positive spiral. Photograph of worms giving a positive reaction to a current of water stirred in the clockwise direction.

FIG. 2. The negative spiral. Photograph of worms giving a negative reaction to a current of water stirred in the clockwise direction. The worms near the center are reacting negatively while those about the outer part of the pan, in the stronger current, are reacting positively.

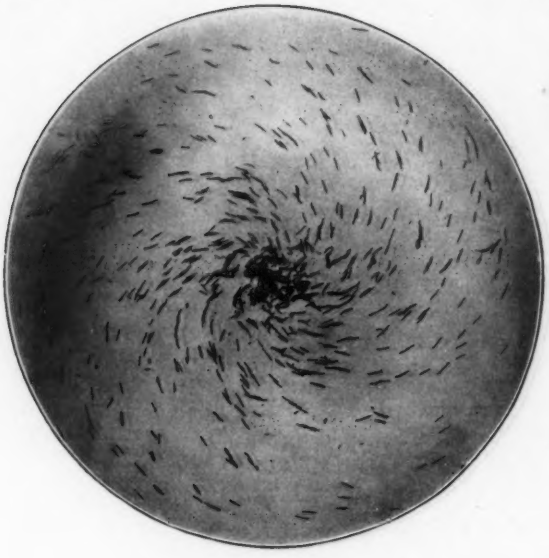

FIG. 1.

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FIG. 2

PLATE II.

FIG. 3. The system of spiral currents. Photograph of pan in which the water has been stirred in the clockwise direction and then drops of carmine have been placed on the bottom at various points about its circumference. The dark lines are streaks of carmine produced by the currents and showing the direction of the currents upon the bottom.

FIG. 4. A positive reaction showing individual variation in the precision of orientation.

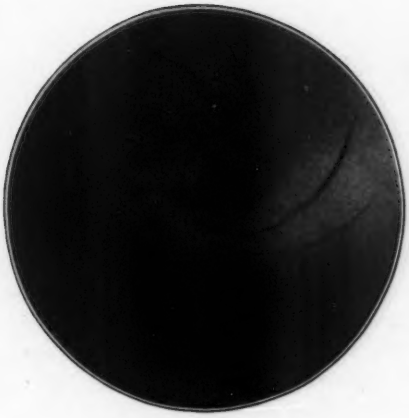

FIG. 3.

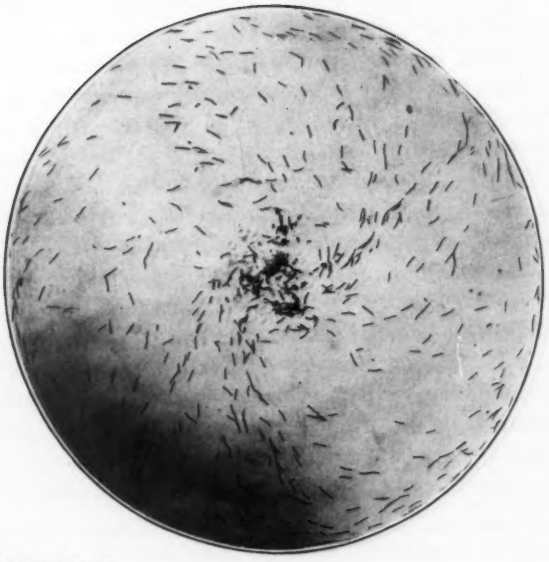

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FIG. 4.

A CASE OF HERMAPHRODITISM IN SPELERPES BISLINEATUS.

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Hermaphroditism among *Anura* seems to be comparatively common. Ecker and Wiedersheim's "Anatomie des Frosches," as revised by Gaupp, devotes several pages to the subject, dividing it into various types, the most common being that in which the glands are essentially male with female elements.

Miss King ('10) in her paper on anomalies in the genital organs of toads, says: "Evidently hermaphroditism occurs much less frequently among the *Urodela* than among the *Anura*, as only two cases have as yet been reported for this group of Amphibians. La Valette St. George ('95) has given a brief description of a case of hermaphroditism in *Triton tæniatus* and Knappe ('86) has noted the presence of a Bidder's Organ in a young salamander; neither investigator gives any details regarding the structure of the ovo-testes in these forms."

Knappe mentions *Triton* also in his investigations but seems to have found a Bidder's Organ only in a two-year-old *Salamandra maculata*. Concerning the frequency of occurrence of hermaphroditic salamanders, La Valette St. George says: "Für die Urodelen liegen, soviel mir bekannt, noch keine Angaben über Zwitterbildung vor.

"Spengel will solche niemals angetroffen haben, obgleich er zahlreiche Salamander und Tritonen zerlegt und allein von Triton cristatus über 100 männliche Individuen untersucht hat."

While collecting data concerning the spermatogenesis and age of attainment of sexual maturity of *Spelerpes bislineatus*[1] I found that a series of sections through the testes of a 46 mm.

[1] Although this species has long been called *bilineatus*, Dr. Green in his original description of it (Jour. Acad. Nat. Sc. Phila. Vol. I, Pt. II, 1818) gave it the name of *Salamandra bislineata*. With the change in generic name the masculine ending was adopted and the species is correctly *Spelerpes bislineatus*.

larva, collected in September, 1913, contained several eggs. The larva was nearly of adult proportions and presumably would have undergone metamorphosis the following summer.

Fig. 1, *B*, is from a drawing of the pair of gonads and kidneys, made with an Abbé camera before imbedding. It reveals the peculiarities of the glands when compared with Fig. 1, *A*, which is a drawing, at the same magnification, of a normal pair of

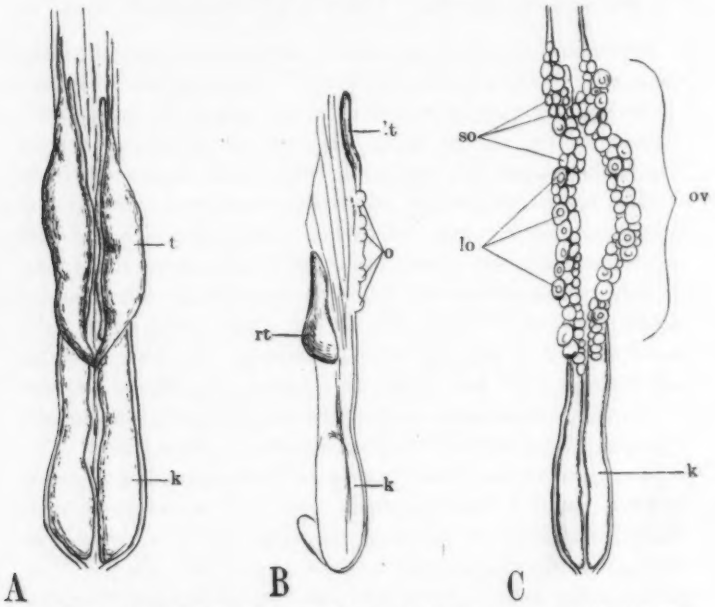

FIG. 1. *A*. Testes and kidneys of normal 48 mm. larva. *B*. Gonads and kidneys of hermaphroditic 46 mm. larva. *C*. Ovaries and kidneys of normal 46 mm. larva. These gonads and kidneys are from larvæ collected in September. Ventral view, × 7½. *k* = kidney, *lo* = largest ova, *lt* = left testis, *o* = ova, *ov* = ovary, *rt* = right testis, *so* = smaller ova, *t* = testis.

testes and kidneys of a 48 mm. larva. The original of Fig. 1, *B*, being merely a hastily made outline drawing and the testes having been very small, I noticed the hermaphroditic condition only on careful study of the sections.

A comparison of Fig. 1, *A* and *B*, shows that, in the abnormal specimen, neither testis is fully developed. The hermaphroditism

is of two types. Macroscopically, the anterior part of the left gonad, which is much reduced in size, resembles the normal testis in texture, though not in shape, while the posterior region is distinctly like an ovary. The larger ova in this latter region, indicated in outline in Fig. 1, *B*, are six in number.

Fig. 1, *C*, is a camera drawing of the ovaries and kidneys of a normal 46 mm. larva, collected in September, showing the arrangement of eggs, for comparison with their arrangement in the posterior region of the left gonad of the hermaphrodite. In each normal ovary the larger ova lie approximately in two rows, one lateral and one medial. A dozen or fifteen on each side are appreciably larger than the others. These larger ones, as may be deduced from a comparison with the ova in specimens of adult female *Spelerpes*, collected in September, are of the size and state of development which indicate that they would normally have been deposited a year from the following spring as the first brood of this individual, while the next smaller ones would have been deposited two years from the following spring.

The larger ova in the left gonad of the hermaphrodite are about the size of the smaller ova in the normal ovaries, though their irregularity in shape, especially where large numbers of eggs are packed together, makes exact measurements impossible. The ova of both the normal specimen and the hermaphrodite have their long axes parallel to the longitudinal axis of the body. This diameter, as measured from sections, is .15 to .20 mm. while the shorter diameter, at right angles to the long axis, averages about .12 mm. The largest ova found in the hermaphrodite are, in development, a year behind the largest ova in a normal female having the same total length, collected at the same time, and therefore, presumably, of the same age. Beside these ova, there are oögonia in the left gonad of the hermaphrodite, similar to those in the normal ovary which have not yet begun to elaborate yolk (Fig. 2). The relation of ova to follicle cells and to peritoneum in the hermaphrodite is like that in the normal ovary.

Although smaller than the right testis, the anterior portion of the left gonad has somewhat the structure of the normal male gland. The elongated mass is composed of too few loulebs to

have the usual arrangement about a collecting duct. The cells, showing no signs of division to form cysts, which is the immediate preparation for spermatogenesis, are not as far advanced in their development as the germ cells of a normal male larva several months younger than the hermaphroditic one. The left gonad shows throughout a retarded development.

The right gonad, on the other hand, although smaller than the normal testis of an individual of the same size, seems to

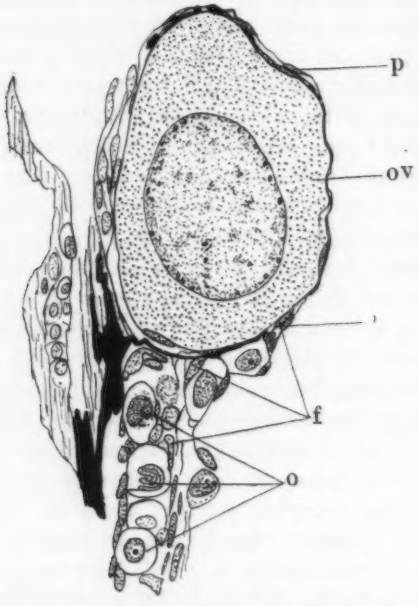

FIG. 2. Horizontal section through posterior region of left gonad of hermaphrodite, × 280. *f* = follicle cell, *o* = oogonium, *ov* = ovum, *p* = peritoneum.

be, in general, normal in structure and in its cellular development. However, it shows another sort of hermaphroditism. Two ova are, in this case, found in the otherwise apparently normal testis, each one completely filling one lobule, which would normally contain a large number of male cells (see Fig. 3). These ova are about two thirds the size of those found in the right gonad. All the lobules are divided into cysts, with the excep-

tion, of course, of the two which contain a large ovum apiece. Most of these cysts are composed of spermatogonia of the last generation, having round or oval nuclei with the chromatin arranged in an irregular network. Nucleoli appear in a few cells of one section. The spermatogonia resemble Kingsbury's Figs. 1 and 2 ('02). In a few cysts, all the cells are undergoing mitosis. These do not resemble the maturation mitoses described for *Desmognathus*, and are probably spermatogonial divisions.

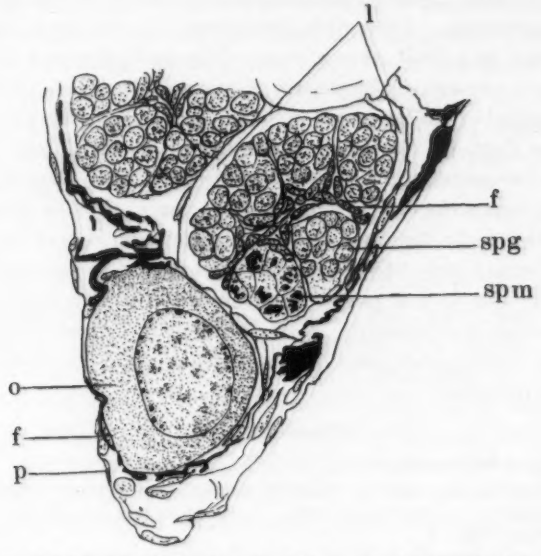

FIG. 3. Horizontal section through right gonad of hermaphrodite, × 280. *f* = follicle cell, *l* = lobule, subdivided into six cysts of spermatogonia, *o* = ovum, *p* = peritoneum, *spg* = spermatogonium, *spm* = spermatogonial mitosis.

In other cysts the nuclei are in the "contracted" condition, described by Kingsbury (Fig. 18), occasionally occurring in *Desmognathus fusca* at the beginning of the period of growth of spermatogonia into spermatocytes and more commonly, in that species, in the last generation of spermatocytes. Fig. 3 shows an egg filling one lobule and surrounded by follicle cells. In this case, as well as in the left gonad, the relation to follicle cells and to peritoneum is normal. The adjacent lobules show sper-

matogonia, grouped in cysts, surrounded by long follicular cells. The cells of one cyst are undergoing spermatogonial mitosis. A great deal of pigment from the peritoneum appears in this section which was cut near the surface of the testis.

The germ cells of the right gonad seem to have reached a normal degree of development. The individual, collected in September, with a total length of 46 mm., would have undergone metamorphosis during the following summer. It is probable that the male *Spelerpes* attains sexual maturity in the fall after metamorphosis. Then this individual should have reached maturity in a little over a year. The spermatogonia are just ready to commence the process of maturation. This process in *Desmognathus* takes about a year, while Kingsbury's observation that, in *Spelerpes*, regeneration of the lobule begins before the spermatozoa leave it, indicates that possibly the process of spermatogenesis requires even more time in *Spelerpes*. Then it may be supposed that these male germ cells of the right gonad would have become ripe spermatozoa at the normal time. The two ova in this gonad, however, are at least a year behind the normal development. In the left gonad, in which there is a greater abnormality, the development of both kinds of germ cells is retarded.

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NOTE ON THE NATURE AND SOURCE OF "PURPLE X."[1]

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In the summer of 1913, Dr. O. C. Glaser ('14) found (1) that if a suspension of *Arbacia* sperm be boiled it turns purple, (2) that this purple color disappears if the boiled suspension be allowed to stand over night, and (3) that initiation of development in *Arbacia* eggs, either by sperm or by egg secretion, can be inhibited by the addition to the eggs of this boiled suspension *so long as it is purple.* This purple substance, he provisionally designated as "purple x."

I. Source of "Purple X."

In 1914, while working with various inhibitors of fertilization in *Arbacia*, I wished to use "purple x" and found that it did not always appear when *Arbacia* sperm was boiled. This naturally led to a search for the source of the substance. Sperm was obtained from a large number of males. Each one was rinsed in fresh water, and after the peristome was cut, placed aboral side down in a clean dry watch-glass, until the seminal fluid had been freely shed. A portion of the sperm from each animal was then mixed with filtered sea-water and examined under the microscope and a second, larger portion of each was boiled. It was found that, as a rule, the suspensions which showed fewest foreign cells—blood-cells or fragments of organs—were least likely to form "purple x." Suspensions which appeared, under the microscope, fairly free from foreign cells, could be depended upon to give a colorless filtrate when boiled.

What, then, is the impurity which produces "purple x"? By a series of experiments properly checked and repeated, I was able to eliminate successively, the filtered perivisceral fluid (serum), the blood clot which had been washed in sea-water

[1] From the Marine Biological Laboratory at Woods Hole and the Zoölogical Laboratory of the University of Michigan.

to free it from serum, fresh fluid containing both serum and blood cells, pieces of the alimentary tract, and mesentery. If a piece of fresh mature testis be boiled in sea-water, in sea-water plus sperm, or in distilled water, a purple compound is formed. This color can also be obtained by treating the testis with strong alcohol, which, as observed under the microscope, turns testis cells from greenish brown to purplish. Whether or not this would happen with immature testes could not be determined, since the experiments were carried on during the breeding season.

The sperm from one male, when examined under the microscope, showed the presence of a few small pieces of testis, and, when boiled, or treated with alcohol, turned purple. The testes were then washed in running sea-water for about three hours, during which they were occasionally pinched with forceps to help free them from sperm. When this was apparently all washed out, a portion of the testis was boiled in sea-water, without giving a purple color. Treating with absolute alcohol, boiling in distilled water, and boiling in sea-water plus fresh sperm also failed to bring out any purple tinge from pieces of the washed testes. The rest were then left standing over night in a finger-bowl of sea-water. After twenty-four hours, it was observed that they tinged the water slightly but distinctly purple.

It seems clear, then, that "purple x" arises from the fresh testis, or from a reaction between testis and sperm. It still remains to be learned whether or not it may be obtained from the ovaries also.

II. Chemical Nature of "Purple X."

The best known pigment of sea-urchins is echinochrome, whose chemical reactions are well established. It may be obtained from the red blood cells by laking with distilled water, by extracting with alcohol, chloroform or ether. The neutral extracts are a cherry red, the addition of a small amount of NaOH turns them yellowish, and acidulating with HCl produces a red-yellow color (MacMunn, '85).

Echinochrome was extracted from the washed blood clot by laking with one volume of distilled water. An equal amount of "double sea-water" (sea-water boiled down to one-half its

original volume) was then added so that the salt content might equal that of the solution with which it was to be compared. A series of experiments was then run in duplicate, using this echinochrome on the one hand, and "purple x" on the other. The addition of NaOH and HCl to echinochrome gave the results described by MacMunn. No visible change could be obtained in the "purple x," however, by the addition of either reagent. Thus it was established that, whatever the chemical nature of "purple x" may be, it is not identical with echinochrome.

III. Physiological Effect of Boiled Sperm.

The addition of boiled sperm suspension to eggs, in *Arbacia*, causes the jelly surrounding the egg to swell, as can be demonstrated by putting them into India ink. It also becomes more sticky. As a result of this, the eggs adhere to each other and to the bottom of the dish. When fresh spermatozoa are added, many get caught in the jelly and form "halos," but some are able to penetrate to the eggs, so that fertilization is not inhibited.

However, when "purple x" is present in the boiled sperm, fertilization by fresh sperm and auto-initiation by egg-secretion is inhibited, as shown in the following table. The percentages were all obtained by counting 200 or more eggs, and a vertical column represents eggs from the same female, treated in different ways.

	Per Cent. of Eggs Divided.						
	A	*B*	*C*	*D*	*E*	*F*	*G*
Eggs without sperm	0	0	0	0	0	0	0
Eggs + sperm	84	72	73	72.3	Polyspermy		
Eggs + boiled sperm (colorless) + fresh sperm		68	55	76.7	82		
Eggs + boiled sperm (purple) + fresh sperm	0			26.6	39		
Eggs + egg secretion + hypertonic after-treatment						43	56.9
Eggs + egg secretion + boiled sperm (purple) + hypertonic after-treatment						0	1 ±

University of Michigan.
March 17, 1915.

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ARE FUNCTION AND FUNCTIONAL STIMULUS FACTORS IN PRODUCING AND PRESERVING MORPHOLOGICAL STRUCTURE?

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Since the days of Lamarck the attempt has often been made of explaining the genesis of the morphological structure of organisms through the theory of adaptation. A special form of this theory is that of "functional adaptation" which was formulated under this name by Wilhelm Roux about 1880, and was later elaborated by that investigator in an extremely extensive and thorough manner.

The most striking organic structures are those which like the bones seem to be constructed on a definitely purposeful plan, offering the largest amount of strength with the smallest amount of material. Other organs, such as the muscles, increase in size as a result of increased function. Roux named this phenomenon "functional adaptation," while the structures underlying this principle he described as "functional structures." He made a number of exceedingly careful anatomical studies of such "functional structures." Endeavoring to explain the genesis of such seemingly purposeful structures from a purely mechanical standpoint, he found that they possessed exactly that construction which was to be expected from a mathematical calculation based on the principle of functional adaptation.

In order to make clear the development of functional adaptation, Roux fell back upon the most primitive particles of living matter. In his opinion some of these particles have been adapted to respond to functional stimuli, that is, they show a greater tendency to proliferate in the presence of functional stimuli than in their absence. Thus, those elements which were subjected to stimuli soon predominated over those which were not thus exposed.

If functional structures consisted of such particles, they would

show the following characteristics: first, enlargement (functional hypertrophy) with increased stimulus; second, atrophy from inactivity upon cessation of the stimulus; third, regeneration of organs of functional structure to the condition of the original structure would only be possible in the presence of function and functional stimuli (functional regeneration); and fourth, the successful transplantation of functional structures would only be conceivable if after transplantation the organs involved were supplied with an appropriate amount of functional stimulus. The principles, therefore, necessary for an acceptance of the theory of functional adaptation are: functional hypertrophy, atrophy from inactivity, functional regeneration, and functional transplantation. (Various other factors which are similarly instrumental in this connection will be dwelt upon more extensively in a subsequent paper.) Should it now appear that these essential principles in so-called functional structures are nonexistent, the theory of adaptation would then fail to adequately account for so-called functional structures.

Before giving the results of my experiments I wish to say a few words about the general value of Roux's theory of functional adaptation.

This theory at a cursory glance has many merits, and at the time when first introduced by Roux it marked a substantial advance, inasmuch as it showed that it was possible to produce highly purposeful structures through the influence of purely mechanical principles. On this basis the theory has become a material factor in connection with the investigations and studies of many workers in the realms of pathology and physiology.

Roux's theory, however, has great disadvantages, one of which is its extremely complicated and extensive terminology. It is probably owing to the extreme obscurity of the doctrine that comparatively few investigators, apart from the originator of the theory, have familiarized themselves with it and fully understood its principles. As a result of this practically the only valuable work along this line has been carried on under the direct control of Roux himself, while in contrast with the comparatively scanty publications emanating from Roux's laboratory an amazingly voluminous mass of literature has been supplied

by outside writers, all of whom were under the impression that they were contributing something to Roux's theory, but who in reality had hardly grasped more than a few but imperfectly comprehended terms connected with the essential problem. These for the most part misleading publications have caused more error than progress in experimental work. I shall endeavor to prove this point in a subsequent and more extensive article.

A second disadvantage of Roux's theory is the fact that the extent of the field in which it is applicable becomes more and more restricted with the increasing number of "experimental" investigations bearing directly upon this problem. Consequently, in course of time the phenomena which are not in harmony with this principle of functional adaptation increase in number, although they may be explained, together with other phenomena, from another point of view.

However, by far the weightiest objection to the theory is that it threatens to become more and more of a stumbling block to workers who are setting out to investigate the problems of organic and inorganic life from a common viewpoint. Nowadays theories such as that of function and functional stimulus can hardly be reconciled with a chemico-physical view of the life processes; for the whole underlying principle of the theory of adaptation does not lend itself to methods of measurement. For this reason a detailed revision of the theory of functional adaptation has become necessary and will be published in a later communication.

In the present paper I shall report on the results of a few experiments which I began five years ago with the above mentioned object in view. As they are at the present moment sufficiently advanced to allow of a survey of the whole point at issue and to show that they are qualified to throw some light on the problem of functional adaptation, a preliminary discussion of these experiments may be warranted.

The experiments in question were performed on the transplanted eye of *Salamandra maculosa*. The eye of a larva was transplanted into the neck of another larva, where after a few days' partial or complete disappearance of the retina resulted, ending finally in complete degeneration. *The remarkable fact*

was that this degeneration was followed later by a complete regeneration of the transplanted eye, even in the dark.

My experiments were conducted with the following considerations in view. The retina is one of those structures which according to Roux's definition should be called a functional structure. But in accepting this definition one need not necessarily assume that these structures are the result of functional stimulus, for such structures may have been predetermined in the embryonic stage. They develop independently of functional stimulus until they reach the third period—the so-called functional period.[1] Functional structures can only exist during this period if they are supplied with a sufficient amount of functional stimulus; otherwise they undergo atrophy through inactivity. From Roux's point of view it would appear probable that the morphogenesis of the eye of *Urodela* is only partially determined by inheritance, and in accordance with this determination it would reach the same stage of development as that attained by *Proteus anguineus*. The further development of the eye, comprising the formation of the rods and cones, that is the true functional parts, would be brought about by the penetration of the rays of light through the skin of the salamander, to its ovaries, and would therefore be the outcome of functional stimulus. Secerov[2] asserts that the skin of *Salamandra maculosa* permits of the penetration of approximately 1/173 of the quantity of light in which the animal lives.

The eyes of *Proteus*, which inhabits dark caves, must therefore remain in the primitive *Proteus* stage, according to the statement of Kammerer,[3] who believes he has demonstrated that further differentiation can only occur if *Proteus* be kept in the light. Thus, he ascribes the process of full differentiation

[1] See, for example, Roux, "Die Entwicklungsmechanik, ein neuer Zweig der biologischen Wissenschaft," Vorträge und Aufs. über Entwickl.-Mech. d. Organismen. 1905, Heft I., p. 94, note 11. Also Roux, "Die vier Hauptperioden der Ontogenese, sowie das doppelte Bestimmtsein der organischen Gestaltungen," *Mitteilung der naturforschenden Gesellschaft*, Halle a.d.S., 1911, I., p. 1.

[2] Secerov, S., "Die Umwelt des Keimplasmas. II. Der Lichtgenus im Salamandra-Körper," *Arch. f. Entwcklungsm.*, 1912, XXXIII., 682.

[3] Kammerer, P., "Experimente über Fortpflanzung, Farbe, Augen und Körperreduktion bei *Proteus anguineus* Laur. Zgl. Vererbung erzwungener Farbveränderungen." III. Mitteil., *Arch. f. Entwicklungsm.*, 1912, XXXIII., 349.

of the eye in the case of several *Proteus* which were kept in the light, directly to the influence of function, and Roux is apparently of the same opinion. This point will be discussed by the writer in a later paper.

My own observations on the transplanted eye of *Salamandra* soon convinced me that this case lends itself very well for the test of the theory of functional adaptation.

First of all I severed the optic nerve, a procedure which according to general opinion should induce permanent degeneration of the retina as a result of the eye becoming isolated from the brain. In all previous operations of this nature, where, however, the eye remained in its normal position, a reunion of the amputated stumps of the nerve took place, so that it was natural to suppose that the re-connection of the eye with the brain brought about regeneration of the retina. In my own experiments I obviated the possibility of such subsequent reunion of the eye with the brain by transplanting the eye to an abnormal position (in the neck of the salamander). But regeneration took place in spite of this fact. The point of chief interest, however, is the fact that by means of this operative measure, which, as has been demonstrated in 95 per cent. of the cases, excludes reunion of the eye with the central nervous system, the eye is permanently deprived of functional power.

It is thus obvious that in these eyes no function was possible and the experiment therefore shows that a whole series of phenomena, hitherto designated as cases of functional adaptation, require a different explanation. We will now discuss these phenomena in greater detail.

I. In about a week after transplantation of the eye into the neck of the salamander the retina had degenerated to such an extent that in many cases only the peripheral part of the retina, which was not differentiated in layers, had survived.[1] But in spite of its permanent isolation from the brain and despite the fact that the eye was permanently deprived of function, the retina from this time on began to show signs of regeneration and the transplanted eye began to receive a progressively im-

[1] E. Uhlenhuth, "Die Transplantation des Amphibienauges," *Arch. f. Entwcklungsm.*, 1912, XXXIII.

proved supply of blood, so that in a comparativey short time (from 4 to 6 weeks) it had regained a perfectly normal structure.

II. This regeneration of the transplanted eye even takes place when the organ is deprived of functional stimulus by light. A series of salamanders operated on in the above-described manner, were placed in a dark room where neither red nor white light could penetrate to their eyes; but in spite of this fact the transplanted eyes regenerated and developed a normal retina.

These experiments show that the "quality" of this process, namely, regeneration as such, is independent of any sort of functional influence. We are here dealing with a case of simple regeneration, such as is found in many organs, not with *functional* regeneration, such as we might expect to find in so-called functional structures. Of course this fact does not warrant us in entirely rejecting the theory of functional adaptation, for the possibility must not be ignored that although regeneration occurs in eyes treated in this way as a result of the agency of certain other factors, nevertheless degeneration brought about by atrophy through inactivity might follow later, as a result of the permanent lack of function and functional stimulus, a possibility which would be expected to arise according to Roux's theory.

III. But secondary degeneration as a result of atrophy from inactivity failed to occur in my experiments, even when the eyes were permanently deprived of function, as occurred in the "light" series.

IV. Degeneration similarly failed to occur in the transplanted eyes which were permanently deprived of both function and functional stimulus, namely in the "dark" series. These eyes, although severed from the brain and in permanent darkness, grew and metamorphosed simultaneously with the normal eyes of the hosts.[1]

Up to the present time I have had at my disposal preparations of eyes of the "dark" series which were preserved 15½ months after transplantation; at that time the hosts were about 21

[1] E. Uhlenhuth, "Die synchrone Metamorphose transplantierter Salamanderaugen (Zugleich, Die Transplantation des Amphibienauges II. Mitteil.) *Arch. f. Entwcklungsm.*, April, 1913, XXXVI., 211.

months old, and all the structures, with the exception of the sex organs, were perfectly developed. The retinas of these transplanted eyes were found to be normal in every detail.

In addition to the above I have a preparation of an eye of the "light" series, which was preserved 3 1/4 years after transplantation, at which time the sex organs were also fully developed. According to Roux the eye of an amphibian should by that time already have entered the functional period, as is believed to have been proved in the case of the eye of the *Proteus*. Nevertheless, the old transplanted eyes were also found to be normal, and the functional parts of the retina, viz., the rods and cones, were present and well developed.

The above results, namely, the permanent preservation in a normal condition of transplanted eyes, prove beyond any doubt that the so-called functional structures of the eye do not undergo atrophy through inactivity, even if they are kept under extremely unfavorable conditions and are deprived of all function and functional stimulus.

This fact alone is sufficient to show that atrophy from inactivity, which is one of the fundamental postulates of Roux's theory, is by no means a phenomenon of general occurrence which takes place in all so-called functional structures permanently deprived of functional stimulus, as was supposed to be the case.

As far as regeneration is concerned, the experiments mentioned above only serve to show that regeneration in itself is independent of function and functional stimulus.

V. I am, moreover, able to demonstrate that the "quantity" of the regenerative process in the eyes, viz., the rapidity of this regeneration are not influenced by functional stimulus, viz., light.

A certain number of animals (260 in all) of both the light and the dark series were preserved at certain intervals of time and the transplanted eyes were cut in sections. Eyes preserved at equal intervals of time were then compared with each other. It was found that the transplanted eyes of the same series which had lived on the host for the same period of time may show considerable differences in the rapidity with which they undergo regeneration, even if they are subjected to equal conditions

as far as functional stimulus is concerned. These differences must therefore be caused by other non-specific factors. In order to ascertain how far-reaching is the influence of light, it was necessary to determine the average rapidity of regeneration in every group of eyes of the same series and make a curve for each series. Although these curves are not yet completed, the results thus far obtained show no differences between light and dark series at all. Even the quantity of the regeneration is therefore uninfluenced by light.

From the above data we must draw the following conclusions: *Functional adaptation plays no part either in transplantation or in regeneration of the retina; nor is it a factor which determines either the quality or the quantity of these processes.*

This, of course, does not mean that regeneration or transplantation of the eye cannot be influenced at all by chemical or physical factors. On the contrary, as is shown by the differences between transplanted eyes of the same series, examined at equal intervals of time after transplantation, the quantity of regenerative processes, viz., the rapidity of regeneration are subject to variation by one or more factors. The point of importance is the fact that these factors are not connected with the specific functional stimulus, viz., light. Apparently they are the same factors which also affect the rapidity of the regenerative process of other organs. The factor concerned is probably the length of time since circulation in the transplanted eye was reëstablished.

Hitherto the regeneration of the retina has been considered as being different from the regeneration of the bones. It was supposed that a bone could only regenerate its architectural structure if in use, otherwise the result would not be a normal bone but a disorderly bony mass.

Aug. Bier,[1] however, has shown that even the bones do not regenerate as a mere indefinite mass of bone if kept without functional stimulus, but that on the contrary, in the absence of any such stimulus, they resume their original functional structure to the minutest details. In certain experiments a considerable part of a human tibia was removed. Although these

[1] Aug. Bier, "Beobachtungen über Knochenregeneration," *Arch. f. klin. Chir.*, Dec. 1912, c, 91.

bones were not exposed to any function, the tibiæ after a certain length of time assumed their normal shape and structure, but only if they were supplied with nourishment in a proper way, and if sufficient space was left for them to regenerate the missing part.

The same is true of the tendons. H. Triepel[1] showed that the tendo Achillis of a cat can regenerate only tendon tissue, irrespective of the presence or absence of functional stimuli.

Our own experiments now prove the same principle also in the case of the eye of *Salamandra maculosa;* this organ regenerates its functional structures in the absence of functional stimulus, and furthermore it retains its structure permanently, despite the permanent absence of functional stimulus. For a long time it was believed that a bone only regenerated a structureless mass of callus in the absence of function, and according to this theory it would be assumed that an eye if once degenerated would in the absence of light regenerate undifferentiated retina cells, such as we find in the normal *Proteus* eye. Nevertheless, both eye and bone regenerate the normal and fully differentiated structure, even in the absence of functional stimulus.

We have seen that in my experiments the velocity of the process of regeneration was not influenced by function; and even if this had been the case it could not be used as a proof in favor of functional adaptation. There are a number of well known morphogenetic processes, the rapidity of which can be accelerated by light, although light bears no relation to the function of the developing organ, that is to say, is not a functional stimulus. The most striking experiments made in this connection are those of J. Loeb,[2] in which he showed that the regeneration of the hydrants of *Eudendrium* is impossible in the absence of light. Nevertheless, we cannot call this a case of functional adaptation, because here light is obviously not a functional stimulus. On the other hand, development of the eyes of fish embryos cannot be prevented by the exclusion of the func-

[1] Triepel, H., "Selbstständige Neubildung einer Achillessehne," *Arch. f. Entwcklungsm.*, Aug., 1913, XXXVII., 278.

[2] Loeb, J., "Über den Einfluss des Lichtes auf die Organbildung bei Tieren," *Pflügers Arch.*, April, 1896, LXIII., 273.

tional stimulus. But it is incorrect to ascribe this fact to the assumption that heredity may have fixed this character so far that development of an eye now occurs in the absence of light. For Loeb[1] has shown that it is very easy to prevent the development of these eyes by a number of different means, such as lack of oxygen, which is a non-specific, non-functional factor. Moreover, it is not necessary to point out that the influence of light on a photographic plate has never been considered to be a case of functional adaptation, although the sensitiveness of the plate to light is just as much response to a physical factor as is the regeneration of *Eudendrium* or as might be the variation in the rapidity of eye-regeneration which although not found in our experiments, might possibly have occurred.

The theory of functional adaptation complicates instead of simplifying the problem. What we should emphasize is not the fact that the result of the response to light is different in the case of *Eudendrium* from what it is in the case of the regenerated eye or of the photographic plate, but the fact that these three phenomena all possess a common basis. It is obvious that all three are governed by the same laws, with which we are familiar from our knowledge of physics and chemistry; but these laws are free from such terms as function, functional stimulus, or any other stimulus, or the principle of adaptation. In order, therefore, to obtain a fertile method for attacking the problems confronting us we must constantly bear in mind the fact that the same laws expressed in the same terms can explain both organic and inorganic phenomena.

[1] Loeb, J., "Heredity in Heterogeneous Hybrids," *Jour. of Morphol.*, March, 1912, XXIII., 1.